KIDNEY

A Sensitive Nephrotoxicity Assay of *in vitro* Podocyte Effacement

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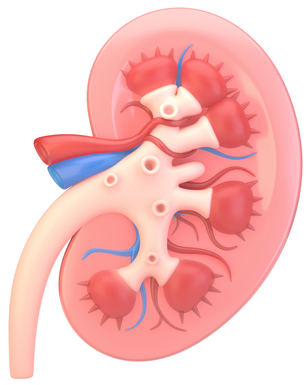


Introduction

Podocytes are specialised epithelial cells in the kidney glomerulus that have long finger-like processes which wrap around blood vessels to form a size-selective sieve (**Figure 1**). This sieve allows ions, water and other small molecules to pass through, while retaining large molecules, including albumin, in the blood. Damage to this glomerular filtration barrier (GFB) due to drug-induced injury or disease, can make the holes in the sieve larger, allowing bigger molecules to pass, and in turn causing damage further down the kidney tubules. However, not all injury causes podocyte loss; often small morphological changes occur where the foot processes retract (effacement). We previously developed a primary podocyte FITC-dextran assay that enabled us to assess drug candidates for their ability to make the holes in the GFB larger, but we now wanted to develop an assay to monitor the small morphological changes; an assay for effacement. For this we used high content imaging.

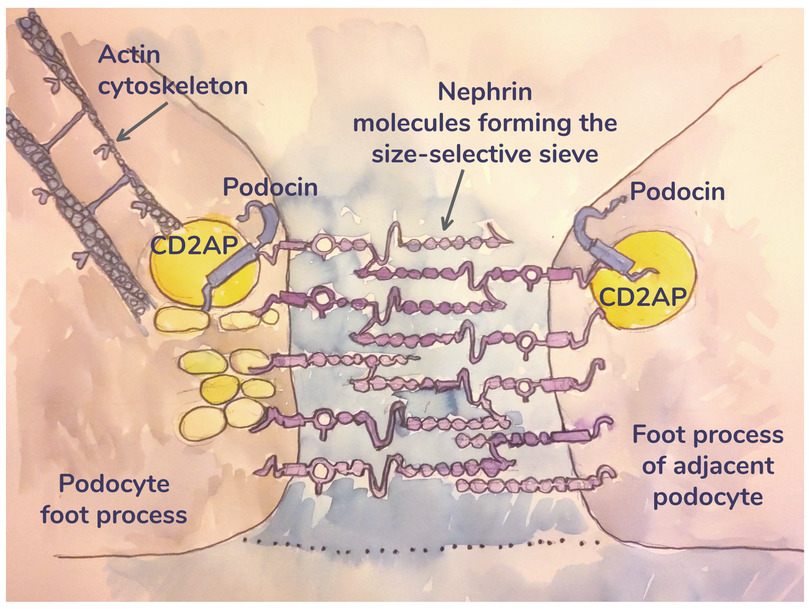


Figure 1: The slit diaphragm structure between interdigitating foot processes: Nephrin, Podocin and CD2AP and other proteins form a size-selective sieve.

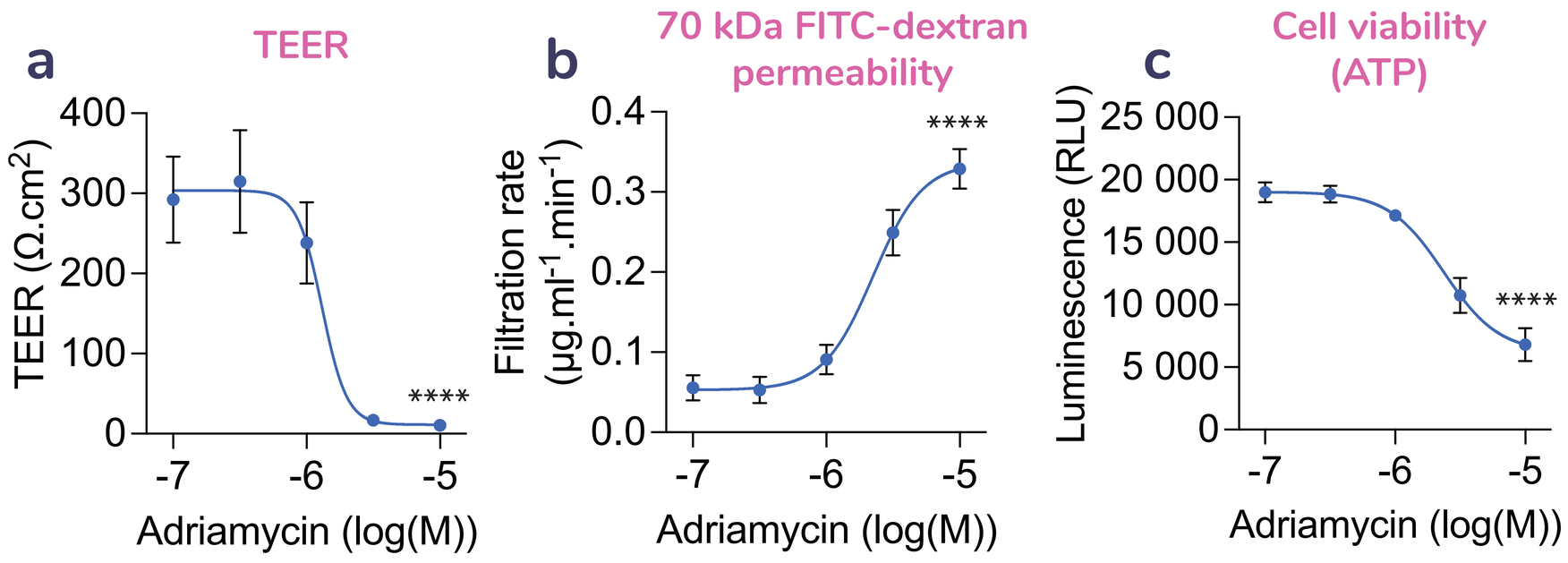
Methods

Primary podocytes isolated from human kidneys were seeded onto 96-well Transwell inserts (Corning #3381) or black-walled 96-well imaging plates (Corning #3904). Transepithelial Electrical Resistance (TEER) was measured using an EVOM2 Voltohmmeter for cells grown in Transwell inserts. When confluent (TEER >100 $\Omega.cm^2$), podocytes in either format were treated with Adriamycin or Tumor Necrosis Factor (TNF) for 72 h. For podocytes in Tranwells, 70 kDa FITC-dextran was applied to the upper compartment and rate of filtration through the podocyte monolayer assessed at 30, 60, 90 mins by performing linear regression analysis. Cell viability was determined using CellTiterGLO (Promega). For podocytes grown in black-walled plates, cells were fixed following treatment and stained with Phalloidin-647 dye or immunostained for CD2AP, Podocin and Nephrin proteins and imaged using the ImageXpress Pico high content imaging system. Images were analysed using Cell Reporter Xpress (CRX) software (Molecular Devices).

Results

We previously developed a podocyte toxicity assay that could be used to test whether drug candidates caused damage to the GFB. This assay measured filtration of 70 kDa FITC-dextran (the approximate molecular weight of albumin).

Figure 2 (right): Adriamycin treatment increases Podocyte monolayer permeability. (a) Adriamycin induces a dose-dependent decline in barrier integrity (trans-epithelial electrical resistance, TEER). **(b)** Permeability to 70 kDa FITC-dextran increases in response to Adriamycin. **(c)** Cell viability decreases in response to Adriamycin indicating that podocyte death and loss likely leads to an increase in barrier permeability (*N*=4).



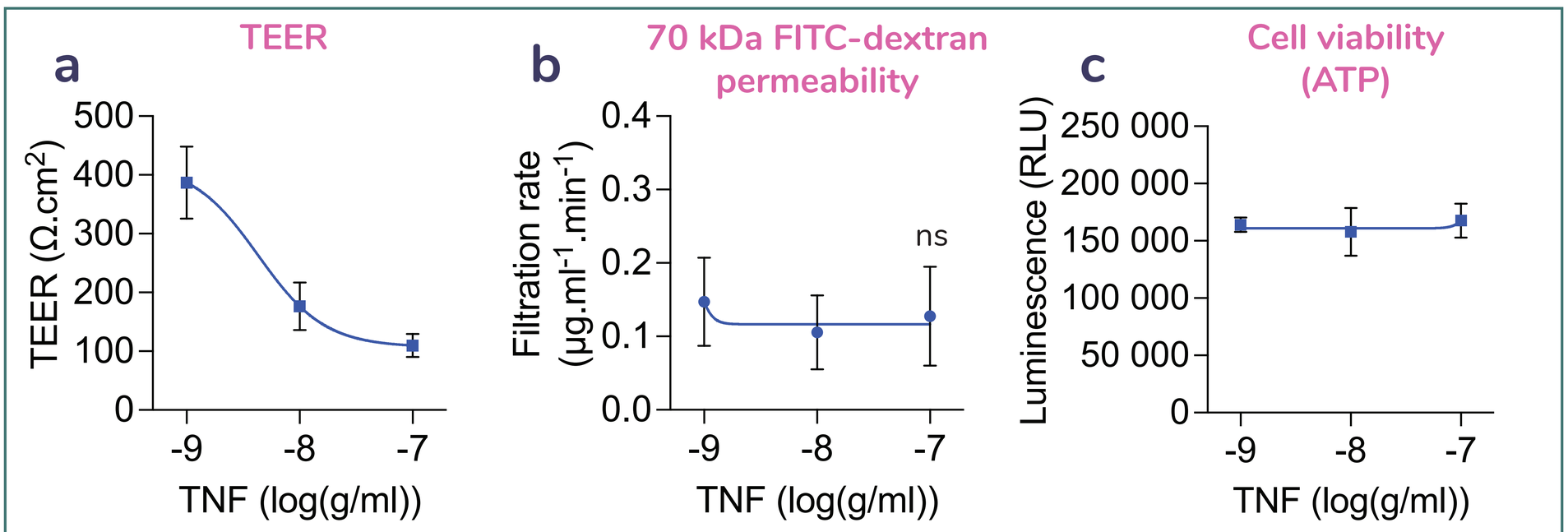


Figure 3: TNF causes a decline in TEER but has no effect on cell viability. (a) TNF promotes a dose-dependent decrease in TEER. **(b)** Podocyte monolayer remains impermeable to 70 kDa FITC-dextran following treatment with TNF. **(c)** Cell viability is unaffected by TNF.

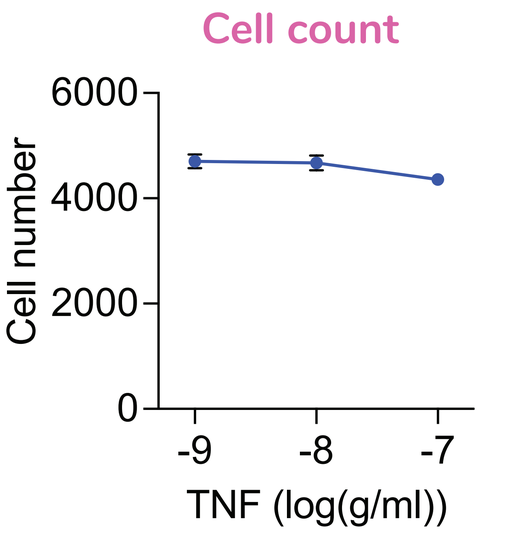


Figure 4: Total cell number is not significantly affected by TNF, indicating that the decline in TEER could be due to morphological changes in the podocytes rather than cell death.

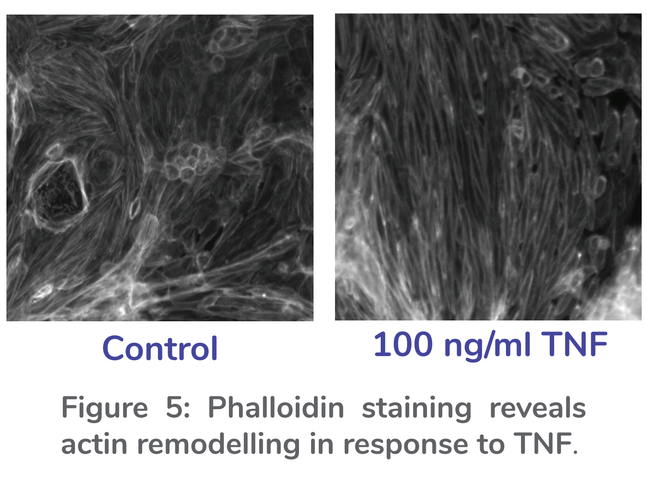


Figure 5: Phalloidin staining reveals actin remodelling in response to TNF.

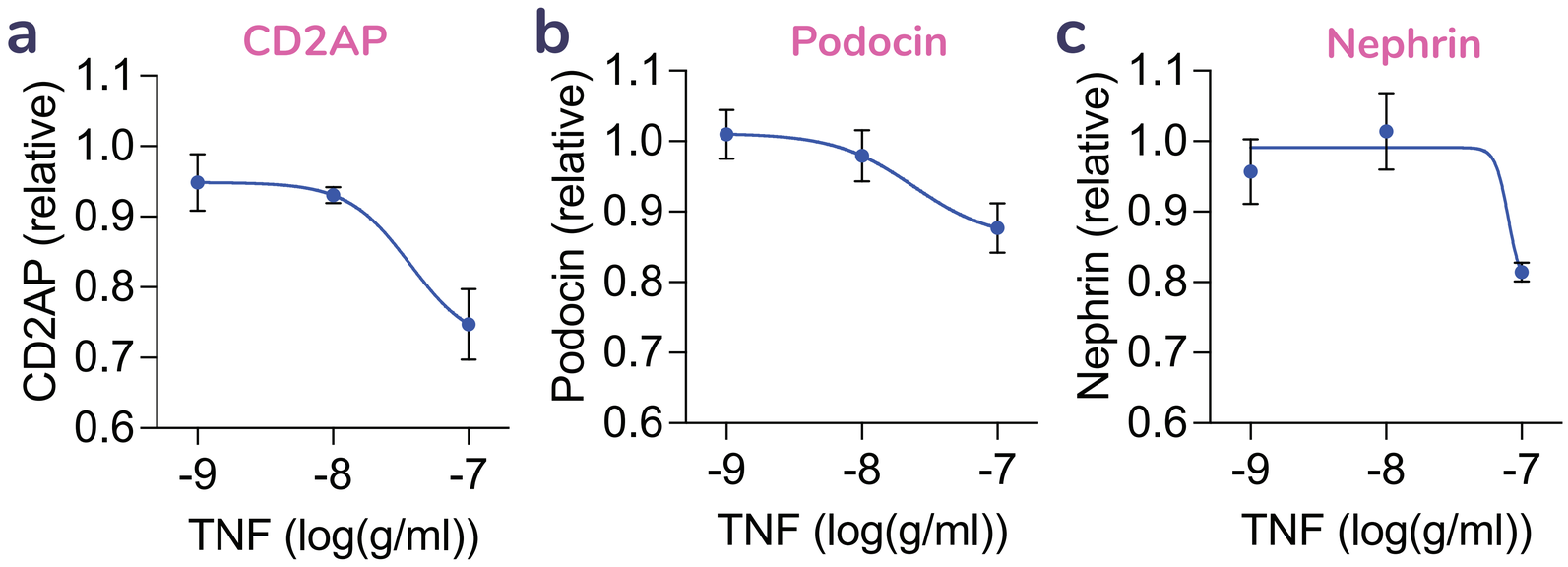


Figure 6: TNF downregulates podocyte marker expression. Since TNF promotes actin remodelling in podocytes, we wanted to see what effect this had on podocyte-specific proteins in the slit diaphragm. We observed that TNF promoted a decline in **(a)** CD2AP, **(b)** Podocin, **(c)** Nephrin proteins by high content imaging. This indicates that TNF promotes podocyte de-differentiation, dismantling of the slit diaphragms and possibly retraction of the podocyte foot processes in response to TNF.

Conclusion

- **Immunostaining and high content imaging for the podocyte proteins CD2AP, Podocin, and Nephrin, reveal small morphological alterations in podocytes that can be used as an early or low level podocyte toxicity assay in drug development.**

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